Pestic. Sci. 1996, **46**, 361–367

Investigations on the Behaviour of Fenpropimorph and its Metabolite Fenpropimorphic Acid in Soils

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(Received 5 May 1995; revised version received 11 September 1995; accepted 31 October 1995)

Abstract: The fate of fenpropimorph and its metabolite fenpropimorphic acid was investigated in a silty sand soil and in a clayey silt soil. In laboratory and field experiments fenpropimorph disappeared without a lag phase. A few days after application fenpropimorphic acid was detected. Additional laboratory experiments with [^{14}C]fenpropimorph emphasized the significance of mineralization and the formation of non-extractable residues.

The determination of soil/water distribution coefficients of parent compound and metabolite yielded a higher leaching potential for fenpropimorphic acid due to its higher polarity. This was confirmed by performing a laboratory column test under worst-case conditions. Under field conditions, however, fenpropimorphic acid was detected only in the superficial soil layers (0–5 cm) of both investigation sites at very low concentrations.

Key words: fenpropimorph, fenpropimorphic acid, degradation, leaching, soil

1 INTRODUCTION

Fenpropimorph [(±)-*cis*-4-[3-(4-*tert*-butylphenyl)-2-methylpropyl]-2,6-dimethylmorpholine] is a systemic fungicide applied against *Erysiphe graminis* DC, *Puccinia striiformis* Westend and *Puccinia recondita* Rob. ex Desm. Its fungicidal activity is based on inhibiting the sterol-Δ^{14}-reductase as well as the $\Delta^8 \rightarrow \Delta^7$-isomerase of fungi.[1–3] This morpholine fungicide is an important alternative to the widely-used azole fungicides which inhibit the sterol-14-α-demethylase, and which are confronted with resistance.[4]

Despite the significance of fenpropimorph in agricultural practice in Germany, its fate in soil has so far only been investigated to a limited extent. In a long-term field study, the residual behaviour of fenpropimorph in top soil of a Luvisol was described by determination of only the parent compound.[5] In a lysimeter experiment, degradation and leaching were also investigated by application of a ^{14}C-labelled fenpropimorph formulation.[6] Besides the parent compound applied, fenpropimorphic acid [(±)-*cis*-4-{3-[4-(1-carboxy-1-methylethyl]phenyl]-2-methylpropyl}-2,6-dimethylmorpholine] and a morpholine ring cleavage product [*N*-[3-(4-*tert*-butylphenyl)-2-methyl]propyl-*N*-(2-hydroxyethyl)amine]were determined as metabolites in the extractable fraction. The mass balance, which was completed by determination of non-extractable residues, revealed losses of about 70% of applied radioactivity, due to mineralization or post-applicative volatilization. However, all residual data gathered in this open system described exclusively the situation at the end of the investigation period and were focused on a strongly sorptive soil type. Consequently, there is no information about dependence of degradation on time and about leaching in a less sorptive soil type.

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Pestic. Sci. 0031-613X/96/$09.00

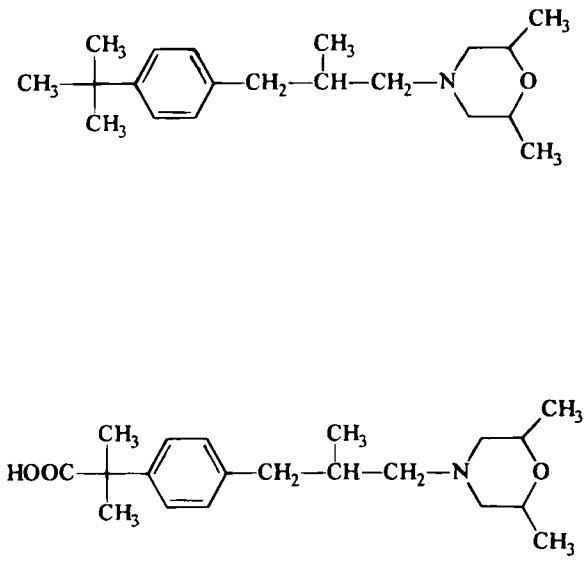

Fig. 1. Chemical structures of (1) fenpropimorph and (2) fenpropimorphic acid.

The objective of the present laboratory and field studies was, therefore, to investigate the residual behaviour of fenpropimorph and fenpropimorphic acid (Fig. 1) after periodical sampling. The concentrations of both compounds were determined in a clayey silt soil as well as in a silty sand soil. In order to assess leaching potential, soil/water distribution coefficients were determined and a column experiment under worst-case conditions was performed. Leaching behaviour was also studied on a Luvisol and a Cambisol under field conditions. Finally, detailed mass balances were determined in laboratory experiments by applying [^{14}C]fenpropimorph.

2 MATERIAL AND METHODS

2.1 Investigation sites

The investigations were performed at two catchment areas of the Special Collaborative Program (SCP 179) 'Water and Matter Dynamics in Agro-Ecosystems' which is established at the Technical University of Braunschweig. The investigation sites were located near Neuenkirchen in the foreland of the Harz Mountains and in Nienwohlde in the Lüneburger Heath, Lower Saxony, Germany. Soil properties are listed in Table 1.

2.2 Laboratory studies

2.2.1 Degradation experiments

Clayey silt soil samples (sieved to <2 mm, 50 g) were fortified with standard fenpropimorph (Dr Ehrensdorfer, Augsburg, Germany) solutions in acetone to give 908 μg fenpropimorph kg^{-1} soil. Samples were incubated in flasks covered with cotton plugs at 24 (±2)°C in the dark. Throughout the incubation period, soil moisture was maintained at approximately 40% maximum water capacity by weighing each flask and adding demineralized water to compensate losses. At 3, 11, 21 and 49 days after application the flasks were closed and frozen at −20°C until analysis. Similar experiments were performed with fenpropimorphic acid (BASF, Limburgerhof, Germany). Samples (50 g) of silty sand soil and of clayey silt soil were fortified with fenpropimorphic acid (750 μg kg^{-1} soil) and residue analysis was carried out 7, 14, 22 and 29 days after application.

TABLE 1
Soil Properties at the Investigation Sites

Investigation sites	*Nienwohlde*	*Neuenkirchen*
Soil type	Cambisol	Luvisol
Soil texture	Silty sand	Clayey silt
Sand [%]	79·7	1·7
Silt [%]	16·0	80·3
Clay [%]	4·3	18·0
$C_{org.}$ [%]	1·2	0·9
pH(0·01 M $CaCl_2$)	6·3	7·3
Maximum water capacity [%]	27·3	33·0

Further laboratory experiments were performed with [*phenyl*-U-^{14}C]fenpropimorph hydrochloride (specific activity: 2·088 MBq mg^{-1}, radiochemical purity: >96%; BASF, Limburgerhof, Germany). Clayey silt soil samples (28 g) were fortified with [^{14}C]fenpropimorph hydrochloride in acetone (841 μg kg^{-1} soil) and mixed thoroughly. The initial activity was 52·2 kBq. Soil moisture was maintained at approximately 40% maximum water capacity throughout the incubation period. According to the OECD guideline for testing of chemicals,[7] glass stoppers of the flasks were equipped with two stopcocks and an internal tube which was filled with 0·1 M potassium hydroxide solution (8 ml) to absorb released [^{14}C] carbon dioxide. The absorption solutions were exchanged every three to four days in order to determine mineralization rates by liquid scintillation counting (LSC). Soil samples were taken after 3, 8, 16, 32, 64 and 102 days and frozen until analysis.

2.2.2 Determination of distribution coefficients

In order to assess potential mobility of fenpropimorph and fenpropimorphic acid in soils, distribution coefficients (K_d values) were determined in batch equilibrium studies with four to six replicates.[8–10] As described by Stalder and Pestemer[11] and by Bunte,[12] demineralized water (10 ml) containing 37 μg of fenpropimorph or fenpropimorphic acid was added to dried soil samples

(50 g) and samples were mixed thoroughly and stored at 8°C for a 24-h equilibration period. Subsequently, demineralized water (60 ml) was added and the samples were shaken for 1 h on a horizontal shaker at 220 rev min^{-1}. The aqueous phase was separated by centrifugation at 4000 rev min^{-1} and analyzed for fenpropimorph or fenpropimorphic acid. The amount adsorbed on soil was determined by the difference between amounts in the added solution and in the final supernatant. K_d values were then calculated as the ratio of the concentration adsorbed in soil to the concentration in the aqueous phase.

2.2.3 Soil column experiment

Leaching behaviour was investigated in a soil column experiment under worst-case conditions.[13] Dried soil (silty <2 mm) was filled into glass columns (50 mm ID, filling height: 30 cm), saturated with demineralized water, and fortified with 500 μg of each compound. The surface was covered with a glass frit and demineralized water (393 ml) was applied over a period of 48 h using a peristaltic pump. Infiltration water was sampled and analyzed. Finally, the soil core was separated into 5-cm layers and analyzed for residues.

2.3 Field studies

In May 1992, Fenpropimorph 750 g $litre^{-1}$ EC ('Corbel'^{R}; BASF, Limburgerhof, Germany) was applied at both investigation sites. In contrast to common agricultural practice, it was sprayed directly onto the soil after reaping of barley and wheat, respectively, using a hand-carried spray applicator equipped with a 229 TS spray nozzle (Gloria, Wadersloh, Germany). The fungicide formulation was distributed on a 100 m^2 test plot at a rate of 1·5 kg AI ha^{-1} in 400 litre water. This doubled application rate was used so as to ensure the determination of fenpropimorphic acid, which was expected to be formed only in low concentrations.

Soil samples were taken in the 0–5-cm layer 7, 21, 42 and 70 days or 6, 13, 27, 48 and 76 days after application to the Neuenkirchen or Nienwohlde site, respectively. At the end of the field study, soil was sampled to 30 cm depth using a soil core drill (Humax, Lucerne, Switzerland) in order to determine leaching tendencies. Soil cores (5·1 cm ID, 30 cm length) were separated into four sections, 0–5 cm, 5–10 cm, 10–20 cm and 20–30 cm. At each sampling time, 20 separate soil samples were combined to a mixed sample. Field moist soil was sieved (<2 mm) and aliquots (50 g) were frozen at −20°C till analysis.

2.4 Analytical methods

2.4.1 Residue analysis

Soil samples were analyzed according to the principles of the DFG S19 multi method[14] and the on-line extraction method reported by Steinwandter.[15] After extraction, gel permeation chromatography and derivatization of fenpropimorphic acid with ethereal diazomethane, both compounds were determined by gas chromatography with mass spectrometric (GC/MS) and specific nitrogen and phosphorus (GC/NPD) detection. All parameters are reported in detail by Dieckmann *et al.*[16] The detection limits were 5 μg fenpropimorph kg^{-1} soil and 10 μg fenpropimorphic acid kg^{-1} soil.

Aliquots of the aqueous soil extracts (50 ml) from batch equilibrium studies as well as from soil column experiments were mixed with sodium chloride (15 g) and extracted with dichloromethane (3 × 50 ml). The organic phase was dried with sodium sulfate, evaporated to dryness and dissolved in acetone. After methylation of fenpropimorphic acid, fenpropimorph and its metabolite were analyzed simultaneously by GC/MS and GC/NPD.

2.4.2 Radiotracer analysis

Potassium hydroxide solutions (2·5 ml) were transferred into LSC vials to determine [^{14}C] carbon dioxide as mineralization product. The scintillation cocktail Aquasafe 500 Plus (10 ml) (Zinsser, Frankfurt, Germany) was added and radioactivity was measured using a Tri-carb 2500 TR liquid scintillation counter (LSC) (Packard, Downers Grove, USA). In order to distinguish [^{14}C] carbon dioxide from further volatile compounds, potassium hydroxide solutions (5·5 ml) were acidified with concentrated hydrochloric acid (1 ml) and purged with nitrogen. The nitrogen stream was transferred into a series of three traps filled with methanol (10 ml), hexane (10 ml) and Oxysolve C-400 (15 ml; Zinsser, Frankfurt, Germany), respectively.

Aliquots of each soil sample (0·2 g) were first combusted using an oxidizer Ox-500 (Harvey Instrument Corporation, Hilsdale, USA) to determine total activity. The [^{14}C] carbon dioxide released was sampled in the scintillation cocktail Oxysolve-C-400 and counted. According to residue analysis, soil samples were extracted with acetone (100 ml; Baker, Deventer, Netherlands) to determine extractable residues. Each suspension was filtered twice using a Büchner funnel and a folded filter, respectively. The scintillation cocktail Quicksafe N (10 ml; Zinsser, Frankfurt, Germany) was added to the filtrate (3 ml) for scintillation counting. Aliquots of the soil extracts were rotary-evaporated and examined using a thin layer chromatography (TLC) scanner (Tracemaster 20; Berthold, Munich, Germany). Silica gel plates (20 × 20 cm) with pre-concentration zone (20 × 3 cm) (Macherey-Nagel, Düren, Germany) were developed either in hexane + acetone (3 + 1 by volume) or in toluene + methanol (7 + 3 by volume). Finally, soil residues were combusted to determine non-extractable residues.

3 RESULTS AND DISCUSSION

3.1 Residual behaviour

In laboratory as well as in field experiments, fenpropimorph disappeared from soil continuously without a lag phase. In the laboratory experiment with the clayey silt soil, the concentration of fenpropimorph decreased from 908 to 471 $\mu g\ kg^{-1}$ within 49 days. This corresponded to a calculated half-life of 58 days.[17] Fenpropimorphic acid was already detected three days after application of the parent compound. Within 21 days the concentration increased to 71 $\mu g\ kg^{-1}$ and subsequently decreased to 42 $\mu g\ kg^{-1}$ soil (Fig. 2).

In the field experiment in Neuenkirchen, residues decreased continuously from 1095 to 251 μg fenpropimorph kg^{-1} soil within 70 days (Fig. 3) and half-life was 36 days. Despite the higher initial concentration, which was due to higher application rates and direct soil treatment, these results were consistent with those from the field study of Ebing *et al.*[5] In that study, however, soil management practices were carried out which resulted in a decrease of fenpropimorph to the detection limit of 5 $\mu g\ kg^{-1}$. The investigation in Nienwohlde showed that, in the 27 days after application, fenpropimorph residues decreased from 1166 to 571 $\mu g\ kg^{-1}$ soil, and the half-life was 47 days. Afterwards, the concentrations were found to be approximately constant and they remained higher than those found in the clayey silt soil.

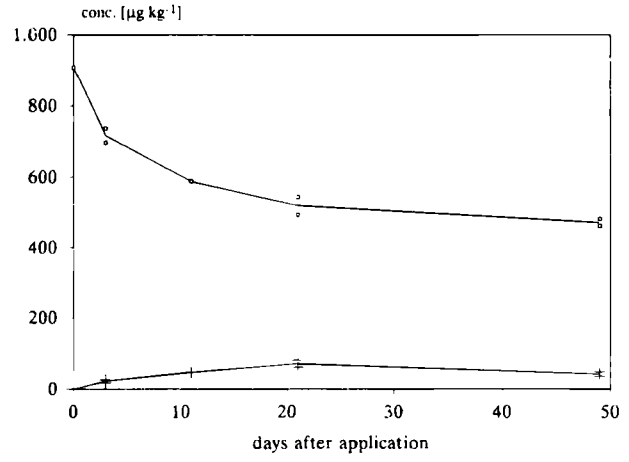

Fig. 2. Concentrations of (□) fenpropimorph and (+) fenpropimorphic acid in the clayey silt soil from Neuenkirchen in laboratory condition. Selected samples were analyzed in duplicate.

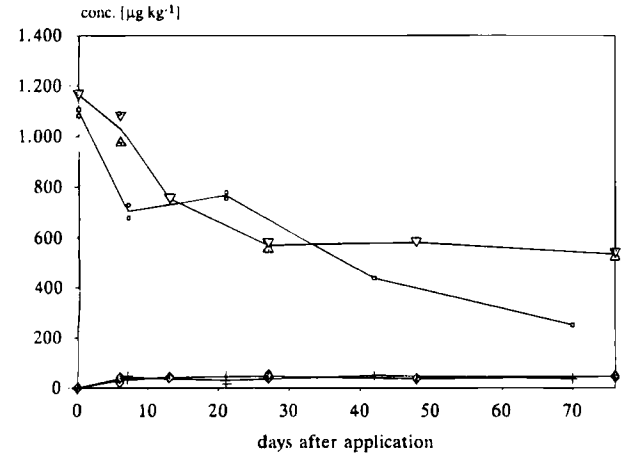

Fig. 3. Concentrations of (□, ▽) fenpropimorph and (+, ◆) fenpropimorphic acid in the clayey silt soil from (□, +) Neuenkirchen and (▽, ◆) in the silty sand soil from Nienwohlde in field conditions. Selected samples were analyzed in duplicate.

Besides a decrease in the active substance applied, fenpropimorphic acid was detected in both soils a few days after the application of the parent compound. The formation of the metabolite confirmed that degradation processes counteract pesticide accumulation in soil. Up to the end of the investigation period, fenpropimorphic acid was determined in the same concentration range. This could indicate a continuous formation and degradation of the metabolite. Results from the laboratory experiments with fenpropimorphic acid itself confirmed a disappearance of this metabolite during the investigation period (Fig. 4).

Because of substantial differences between the concentrations of the parent compound and the degradation product in soil, the disappearance of fenpropimorph could not be explained only by the formation of fenpropimorphic acid. Apart form other metabolites possibly formed, detailed mass balances, which were set up in supplementary batch experiments by applying ^{14}C-labelled fenpropimorph, emphasized the significance of non-extractable residues (Fig. 5). Whereas extractable residues decreased up to the end of the experiment, the non-extractable residues increased rapidly and reached maximum values of approximately 35% after 32 days. At the end of the laboratory trial, they definitely predominated. Results clearly show that

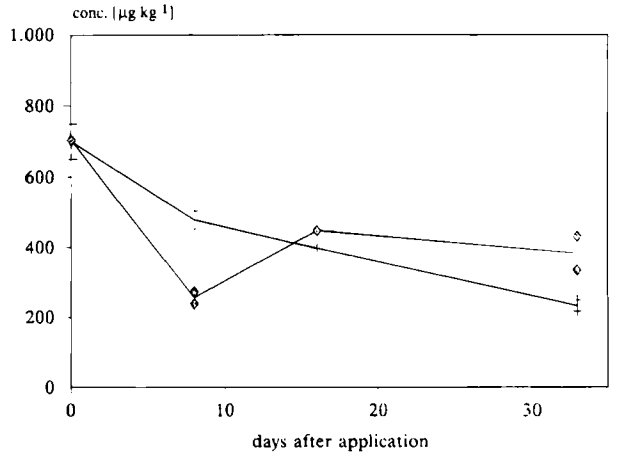

Fig. 4. Concentrations of fenpropimorphic acid in (+) the clayey silt-soil from Neuenkirchen and (◆) the silty sand soil from Nienwohlde in laboratory conditions. Selected samples were analyzed in duplicate.

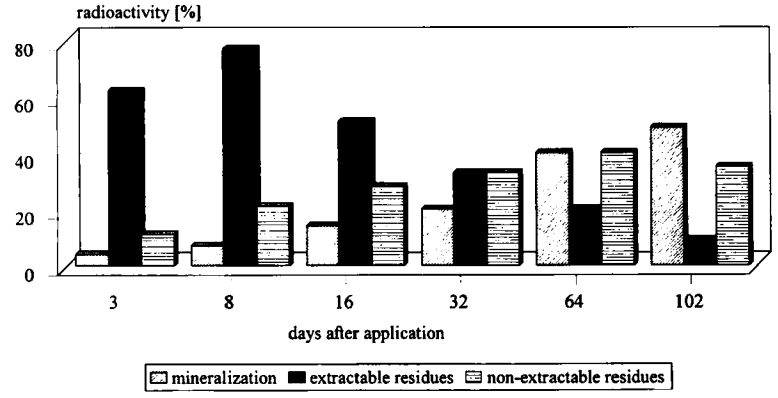

Fig. 5. Mass balance of [^{14}C]fenpropimorph in the clayey silt soil from Neuenkirchen in laboratory condition.

dynamic degradation processes of fenpropimorph continued to mineralization, since [^{14}C]carbon dioxide increased gradually up to 49% of the applied radioactivity. Additional purge and trap trials of potassium hydroxide solutions yielded recoveries of 99% in the [^{14}C]carbon dioxide absorber and confirmed mineralization.

By means of TLC investigations, the extractable residues were separated into fenpropimorph and fenpropimorphic acid. Throughout the investigation period, fenpropimorph predominated in the extracts. This is illustrated by the TLC chromatogram recorded 32 days after application (Fig. 6). These results are in agreement with those of experiments involving unlabelled fenpropimorph. Minor degradation products were detected, but their identification was not confirmed because of the lack of reference standards.

3.2 Leaching behaviour

Leaching tendencies were assessed by determination of distribution coefficients. A strong adsorption of fenpropimorph in top soil was revealed by K_d-values of 79 for the clayey silt soil and 46 for the silty sand soil. For fenpropimorphic acid, coefficients of 1 for the clayey silt soil and 0·5 for the silty sand soil reflected the higher leaching potential due to higher polarity of the metabolite. These results were validated by the column experiment with a disturbed sample of the silty sand soil under saturated water flow. Fenpropimorphic acid was detected in the percolation water with only 0·1% of the applied substance. Analyses of soil layers clearly showed the strong retention of fenpropimorph in the top layer while fenpropimorphic acid was distributed along the soil column with the highest concentrations in the 15–20-cm soil layer (Fig. 7).

According to Fichter and Holden,[18] leaching potential is revealed by parameters as follows: $K_d < 5$, $K_{OC} < 300$, solubility > 30 mg litre^{-1}, soil half-life > 14–21 days and hydrolysis half-life > 175 days. Fulfilling several of these criteria, leaching of fenpropimorphic acid into deep soil layers was expected under field conditions. However, at the end of the vegetation period in 1992, fenpropimorphic acid was only detected in the 0–5-cm soil layers of both soil types at very low concentrations (Fig. 8). The determination of small amounts of metabolite which would be transferred into

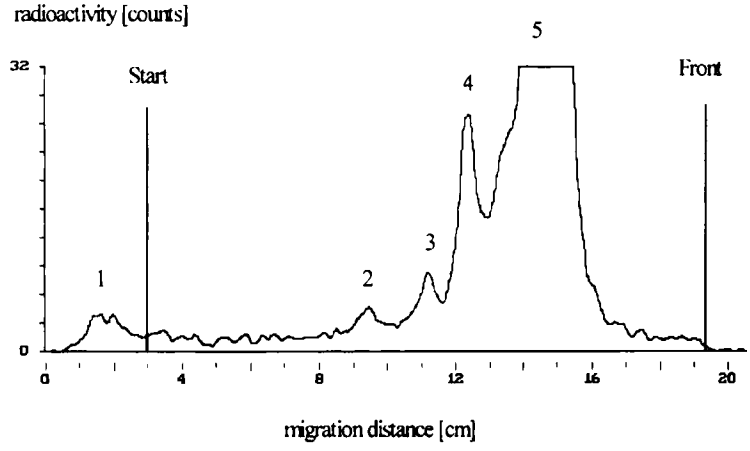

Fig. 6. Thin layer chromatogram of the radioactive compounds extracted from a clayey silt soil 32 days after application (development of silica gel plate with toluene + methanol, 7 + 3 by volume). 1: start spot (area: 1·6%), 2: minor degradation product (area: 1·7%), 3: fenpropimorphic acid (area: 2·3%), 4: *trans*-fenpropimorph (6·9%), 5: *cis*-fenpropimorph (area: 82·6%).

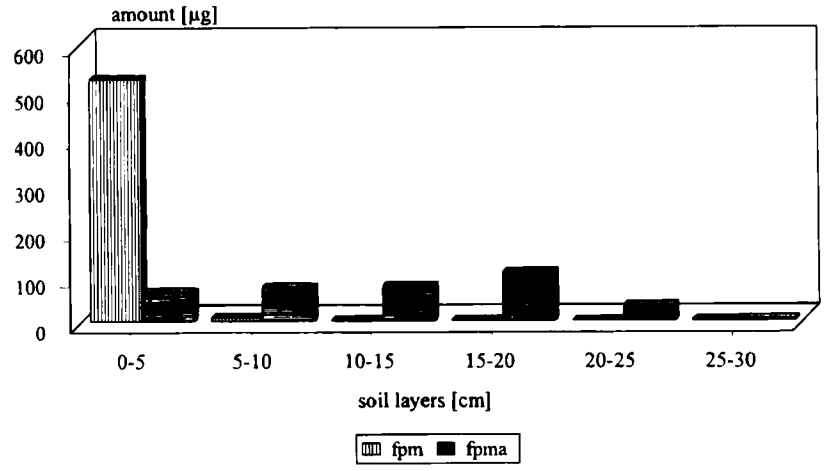

Fig. 7. Concentrations of fenpropimorph (fpm) and fenpropimorphic acid (fpma) in silty sand soil column from Nienwohlde (see Fig. 8).

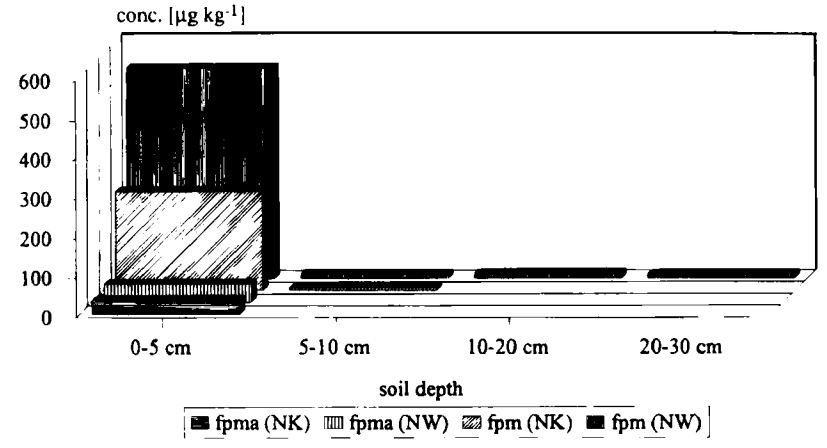

Fig. 8. Leaching behaviour of fenpropimorph (fpm) and fenpropimorphic acid (fpma) in the clayey silt soil from Neuenkirchen (NK) and in the silty sand soil from Nienwohlde (NW) under field conditions.

subsoil might be limited by the analytical limit of detection. The highest concentrations of fenpropimorph were determined in the 0–5-cm layer of both soils. Small amounts of the parent compound were transferred into the 20–30-cm layer only in the silty sand soil. However, a risk of groundwater contamination could not be derived from this investigation. This leaching behaviour is in agreement with the long-term lysimeter experiments of Ebing *et al.*[6] showing that despite additional irrigation, soil contamination was focused on the top soil and no contamination of percolation water was observed during an investigation period of four years.

ACKNOWLEDGEMENT

Authors gratefully acknowledge the financial support by the German Research Society (DFG) due to the Special Collaborative Program 179 'Water and Matter Dynamics in Agro-Ecosystems'. Thanks also to Dr E. Keller, BASF, Limburgerhof, Germany, who supplied some of the reference chemicals.

REFERENCES

1. Bohnen, K. & Pfinner, A., Fenpropemorph, ein neues, systemisches Fungizid zur Bekämpfung von Echten Mehltau- und Rostkrankheiten im Getreidebau. *Med. Fac. Landbouww. Rijksuniv. Gent*, **44** (1979) 487–97.
2. Baloch, R. I., Mercer, E. I., Wiggins, T. E. & Baldwin, B. C., Inhibition of ergosterol biosynthesis in *Saccharomyces cerevisiae* and *Ustilago maydis* by tridemorph, fenpropimorph and fenpropidin. *Phytochemistry*, **23** (1984) 2219–26.
3. Debieu, D., Gredt, M., Gall, C., Malosse, C. & Leroux, P., Mechanism of selectivity to fenpropimorph in *Fusarium genus* and *Pseudocercosporella herpotrichoides*. *Pestic. Sci.*, **43** (1995) 171–3.
4. Alkers, A., Ammermann, E., Buschmann, E., Götz, N., Himmele, W., Lorenz, G., Pommer, E.-H., Rentzea, C., Röhl, F., Siegel, H., Zipperer, B., Sauter, H. & Zipplies, M., Chemistry and biology of novel amine fungicides: Attempts to improve the antifungal activity of fenpropimorph. *Pestic. Sci.*, **31** (1991) 521–38.
5. Ebing, W., Kreuzig, G. & Stemmer, H., Untersuchungen zum Rückstandsverhalten der im Pflanzenschutzmittel-Großversuch Ahlum angewandten Fungizide und Insektizide. In *Auswirkungen eines langjährigen Einsatzes von Pflanzenschutzmitteln bei unterschiedlichen Intensitätsstufen und Entwicklung von Bewertungskriterien. Mitt. a. d. Biol. Bundesanst.*, **295** (1994) 44–69.

6. Ebing, W., Frost, M., Kreuzig, R. & Schuphan, I., Untersuchungen zum Abbau- und Verlagerungsverhalten von Fenpropimorph in einem Lysimeterexperiment. *Nachrichtenbl. Deut. Pflanzenschutzd.*, **47** (1995) 5–7.
7. OECD *Guideline for Testing of Chemicals, Inherent Biodegradability in Soil.* **304A** (1981) 1–11.
8. Oepen von, B., Kördel, W. & Klein, W., Sorption of nonpolar and polar compounds to soils: processes, measurements and experience with the applicability of the modified OECD-Guideline 106. *Chemosphere*, **22** (3–4) (1991) 285–304.
9. Boesten, J. J. T. I., Influence of solid/liquid ratio on the experimental error of sorption coefficients in pesticide/soil systems. *Pestic. Sci.*, **30** (1990) 31–41.
10. Nicholls, P. H., Walker, A. & Baker, R. J., Measurement and simulation of the movement and degradation of atrazine and metribuzin in a fallow soil. *Pestic. Sci.*, **12** (1982) 484–94.
11. Stalder, L. & Pestemer, W., Availability to plants of herbicide residues in soil. Part I: A rapid method for estimating potentially available residues of herbicides. *Weed Res.*, **20** (1980) 341–7.
12. Bunte, D., Abbau- und Sorptionsverhalten unterschiedlich persistenter Herbizide in Abhängigkeit von Flächenvariabilität und Alter der Rückstände. *PhD Thesis*, University Hannover, Germany, 1991, pp. 12–34.
13. Biologische Bundesanstalt für Land- und Forstwirtschaft, Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln. *Versickerungsverhalten von Pflanzenschutzmitteln.* Teil IV, 4-2, 1986.
14. DFG Pesticide Commission, *Manual of Pesticide Residue Analysis.* VCH-Verlag, Weinheim, Germany, 1987.
15. Steinwandter, H., Universal 5 min on-line method for extracting and isolating pesticide residues and industrial chemicals, *Fresenius J. Anal. Chem.*, **332** (1985) 752–4.
16. Dieckmann, H., Stockmaier, M., Kreuzig, R. & Bahadir, M., Simultaneous determination of fenpropimorph and the corresponding metabolite fenpropimorphic acid in soil. *Fresenius J. Anal. Chem.*, **345** (1993) 784–6.
17. Timme, G., Frehse, H. & Laska, V., Zur statistischen Interpretation und graphischen Darstellung des Abbauverhaltens von Pflanzenschutzmittel-Rückständen. II. *Pflanzenschutz-Nachr. Bayer*, **39** (1986) 188–204.
18. Fichter, P. M. & Holden, P. W., A field study to meet United States Environmental Protection Agency regulatory for measurement of movement of pesticides to ground water. *Brighton Crop Protect. Conf.—Pests and Diseases*, **7c** (15) (1992), 853–8.